Editorial

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New therapeutic targets in HCC: Reptin ATPase and HCC senescence

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Cellular senescence is a process characterized by permanent cell cycle arrest. It is the consequence of the finite proliferative capacity of normal cells (replicative senescence) and the response to stress and damage from exogenous and endogenous sources. Several years ago it was shown that senescence is an innate tumour suppressive mechanism associated with the activation of oncogenes, which limits the progression of pre-malignant lesions [1]. As a consequence, escape from senescence is a prerequisite for the progression to malignancy. There are three main mechanisms that trigger cellular senescence and that must be quelled in cancer cells: activation of the p53 pathway, upregulation of the CDKN2A locus, and telomere shortening [2]. Genetic and epigenetic aberrations in any or all of these pathways are common marks of all types of human cancer. However, the senescence resistance associated with transformed cells is reversible [3], and senescence-inducing drugs could represent an ideal opportunity to increase the arsenal of anti-cancer weapons [2]. In the present issue of *The Journal of Hepatology* the work of Ménard et al. describes a new example of tumour progression blockage linked to the induction of replicative senescence in a xenograft model of liver cancer [4].

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and is characterized by a poor prognosis. In many cases no effective therapy at all can be offered to patients with HCC [5]. Surgical interventions such as tumour ablation or transplantation are restricted to a selected group of patients with very specific clinical features, and non-resectable tumours are resistant to conventional chemotherapy. New strategies such as radiotherapy and gene therapy alone or in combination with cell therapy are at early stages of clinical development [6]. Given the survival benefits of the molecular inhibitor Sorafenib in patients at advanced stages [7] the use of single or combined targeted therapies appears as a promising therapeutic alternative. Recent studies demonstrate the existence of an increasing number of pathways that might be altered by drugs in HCC cells [5]. The molecular profiling of HCC and the identification of patient subclasses according to drug responsiveness will lead to a more personalized and effective medicine. As in other types of tumours, aberrations in senescence pathways such as mutations in p53, epigenetic silencing of $p16^{INK4a}$ or induction of telomerase are present in a majority of HCCs [8], representing critical targets for therapeutic intervention.

Telomerase is a ribonucleoprotein complex that adds DNA repeats to the ends of the chromosomes to stabilize them. The upregulation of telomerase expression is a hallmark of cancer cells; therefore, the development of efficient telomerase inhibitors represents a challenge and an opportunity for anti-cancer therapy. Recently it has been shown that the ATPases Pontin and Reptin are essential for the assembling of the telomerase complex [9]. This finding implies that inhibitors of Pontin and Reptin might act as highly effective therapeutic drugs targeting telomerase in human cancer.

Pontin and Reptin are ATP binding proteins that belong to the AAA+ (ATPase associated with diverse cellular activities) family of ATPases which are normally co-expressed and form preferentially heteromeric complexes. They participate in different chromatin remodelling complexes, where they regulate the accessibility of DNA to nuclear proteins in processes such as gene transcription and DNA damage response [10]. Through their chromatin remodelling activity Pontin and Reptin also regulate the functions of the oncogenes c-myc and β-catenin, and have been implicated in cell transformation and metastasis [11]. In addition, both ATPases participate in the assembly and maturation of snoRNPs (small nucleolar RNPs) and as mentioned above in the assembly and function of the RNP complex of telomerase [9].

Using a differential proteomic analysis to look for new HCC targets, the group led by Dr. Rosenbaum previously uncovered the overexpression of Pontin [12] and Reptin [13] in HCC. They demonstrated that Reptin and Pontin expression is associated with poorly differentiated tumours and that both proteins could be considered markers of poor prognosis [12,13]. Immunohistochemical analysis of Reptin and Pontin expression in HCC showed that both proteins are expressed not only in the nucleus but also in the cytoplasm of tumour cells, suggesting possible new cytoplasmic functions related to their pro-tumorigenic activity [12,13]. In the different complexes that they are presented, Reptin and Pontin are found in a 1:1 stoichiometry. In a recent work from this same group, Haurie and coworkers demonstrated the existence of a tight and reciprocal regulation of both proteins [12]. Using specific siRNAs the authors observed that the

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Abbreviations: CDKN2A, cyclin-dependent kinase inhibitor 2A; Dox, doxycycline; HCC, hepatocellular carcinoma; RNP, ribonucleoprotein; SAHF, senescence-associated heterochromatin foci; shRNA, short hairpin RNA; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling.

transcription and translation of the partner protein were not altered. However, the newly synthesized Pontin or Reptin is degraded by the proteasome when either Reptin or Pontin are depleted [12]. Regarding the function of these ATPases in HCC, the same authors demonstrated that both proteins play essential roles in the survival of hepatoma cells. The silencing of either of the proteins in Huh7 or Hep3B human hepatoma cells results in the reduction of cell growth and the induction of apoptosis [12,13]. The role of these proteins in hepatocarcinogenesis is reinforced by the fact that the overexpression of Reptin in Huh7 cells enhances tumorigenicity [13].

All these results together indicate that these proteins indeed play a role in the biology of the HCC cell, and suggest that both ATPases may represent new therapeutic targets in liver cancer. In the current issue of *The Journal of Hepatology* Ménard and coworkers undertake the *in vivo* validation of this hypothesis. To this end they use a doxycycline (Dox)-inducible shRNA system to knockdown the expression of Reptin in already established xenograft tumours in mice. The authors first finely characterize *in vitro* the specificity and inducibility of the shRNA that they subsequently use *in vivo.* They show that the inhibitory effect on cell growth observed upon Reptin-shRNA transfection in Huh7 cells is reversed or rescued after transfection with a shRNA-resistant Reptin construct. Confirming their previous results [12,13], they demonstrate that Reptin knockdown in Huh7 and Hep3B cells arrests cell growth and induces apoptosis. In agreement with previous observations from other groups, Ménard and coworkers confirm that Reptin silencing in hepatoma cells significantly reduces telomerase activity and induces senescence.

In vivo, the authors use these engineered Huh7 and Hep3B human HCC cell lines to induce subcutaneous tumours in NOD/SCID mice. In order to evaluate the effect of Reptin silencing in a therapeutic situation, they administered Dox, to knockdown the expression of Reptin, to mice that harboured established tumours. Upon Dox administration tumour growth not only stopped but even regressed in mice expressing the Reptin specific shRNA. This effect was associated with a significant inhibition on Reptin expression and decreased cell proliferation, but not with a higher level of apoptosis quantified as TUNEL-positive cells. However, the blockage of tumour growth upon Reptin silencing was associated with the induction of senescence, as shown by the increase in senescence markers such as acid β-galactosidase staining and senescence-associated heterochromatin foci (SAHF).

These results present Reptin as a new therapeutic target in HCC and the use of specific Reptin/Pontin ATPase inhibitors as an encouraging alternative for the treatment of cancer in general. However, as the authors discuss, the evidence suggesting that the ATPase activity of Reptin is required in oncogenesis is only circumstantial and further experiments are required to clarify this point.

The work by Ménard and coworkers also raises other interesting questions to be solved. Replicative or telomere-dependent senescence has been linked to the induction of a DNA damage response and the activation of the p53 pathway. However, the two hepatoma cell lines used in the present study are devoid of p53 activity, indicating that alternative pathways are involved in Reptin depletion-mediated induction of senescence. As previously mentioned, in tumour cells, including HCC cells, Reptin accumulates both in the nucleus and in the cytoplasm [11]. Interestingly, the authors observed that cytosolic Reptin was preferentially depleted upon Reptin-shRNA induction, and that the cytosolic downregulation of Reptin was accompanied by a clear inhibition of tumour growth. The reason for this preferential downregulation of extranuclear Reptin is currently unknown; however, these findings are suggestive of a pro-tumorigenic effect of this protein in the cytosolic compartment that warrants further investigation. Although cellular senescence is a cytostatic process, tumour regression occurs. The authors explain this effect as a result of the induction of apoptosis as a secondary event; however, other authors associate tumour clearance with the cooperation between cellular senescence and the evoked inflammatory response [3]. To gain further insight into these and other aspects of Reptin contribution to liver cancer, it would be interesting to examine the behaviour of liver-specific Reptin knockout mice undergoing chemically-induced carcinogenesis.

In summary, the work of Ménard and coworkers uncovers novel targeted therapeutics to re-engage the disrupted senescence program in liver cancer.

Conflicts of interest

The author declares no conflicts.

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